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DEPARTMENT OF
FISHERIES AND WILDLIFE
WESTERN AUSTRALIA

REPORT Nº 63

Published by the Director of Fisheries and Wildlife, Perth,
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Assessment of Artificial Feeds for Battery Culture of a Freshwater Crayfish, Marron *(Cherax tenuimanus)* (Decapoda: Parastacidae)

BY

DR N. MORRISSY

PERTH
WESTERN AUSTRALIA

1984

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Department of Fisheries and Wildlife
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ASSESSMENT OF ARTIFICIAL FEEDS FOR BATTERY CULTURE OF A FRESHWATER CRAYFISH, MARRON (*CHERAX TENUIMANUS*) (DECAPODA: PARASTACIDAE).

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1984

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ISSN 0726 0733
ISBN 0 7244 9317 4

34179—1

AUTHOR'S NOTE

1. Work leading to the contents of this paper was funded by the Department of Science and Technology under Section 39 of the Industrial Research and Development Incentives Act 1976.

2. Title to the results rests with the Commonwealth.

3. Copyright subsists with the Commonwealth.

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ASSESSMENT OF ARTIFICIAL FEEDS FOR BATTERY CULTURE OF A FRESHWATER CRAYFISH, MARRON (*Cherax tenuimanus*) (DECAPODA: PARASTACIDAE).

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ABSTRACT

Performance of juvenile crayfish, 0.1-5 g, on a variety of commercial crustacean feeds was tested under conditions of individual housing ("battery culture"), where sources of additional feed were minimized (detritus), or eliminated (cannibalism). Survival was close, or equal, to 100% over 50 day test periods, a reflection of optimal battery conditions rather than feeding, since survival over 100 days for an unfed (control) group was also 100%. No ecdysial mortality, or trauma, occurred with crayfish, up to 10 g, in compartments of area 75 cm^2. Growth performance in tests was rated by separate ratios for ecdysial increment and frequency, relative to the maximum growth rate of the species recorded in previous studies. Ecdysial weight increment approached the expected 52% on feeds yielding more than one third of the maximum ecdysial frequency. Ecdysial frequency on commercial and experimentally bound and enhanced (vitamins, protein level) artificial feeds was less than 50% (7-44%) of the maximum rate. Exoskeletal pigmentation and thickness and appetite declined over successive ecdyses. These symptoms and tests yielding higher ecdysial frequencies on a standard natural diet of earthworms, or detrital supplemented artificial feed, indicated that the artificial feeds were nutritionally deficient, or became so rapidly in solution. This problem is considered to exist for benthic decapods in general, because of their feeding behaviour, and appears not to have been resolved by more than a decade of intensive research, worldwide.

I INTRODUCTION

Culture of commercially important benthic crustaceans, such as penaeid and palaemonid prawns, homarid lobsters and freshwater crayfish, on artificial (formulated, compounded) feeds is preferable, ultimately, to the use of natural foods (e.g., Wickins and Beard 1978). Successful formulation of such rations for finfish, over the past three decades, has spurred commercial production greatly, notably for salmonids (Millikan 1982). Application of similar feed technology to crustacea over the past decade, or so, has been unsuccessful, except for larval prawns (Kanazawa et al 1982), despite numerous studies (Conklin 1980, New 1980). Current culture of prawns and crayfish relies primarily on natural food chains in earth ponds, enhanced and supplemented by agricultural by-products, or stock feeds (Goyert and Avault 1977, Morrissy 1979, Mills and McCloud 1983). There appears to be some doubt over whether

more costly, formulated, commercial, crustacean rations offer any extra advantage (Fair and Fortner 1981, c.f. Maguire and Hume 1982) and they are insufficient as complete substitutes for traditional natural feeds still necessary at higher levels of production (e.g., Kuruma prawn farming in Japan). However, the "crustacean feed problem" has been most acutely confronted, as a major obstacle to any commercial development, in battery culture of homarid lobsters where the lobsters are individually housed and the sole source of nutrition in bare modules is, perforce, the artificial ration supplied (Richards and Wickins 1979, Van Olst et al 1980). Despite intensive research effort, mainly on *Homarus americanus*, growth of lobsters has been poor on artificial diets (Conklin 1980), until recently (D'Abramo et al, 1981).

In contrast to finfish, which feed to demand at the water surface and ingest pelletted feeds intact, the dilatory and masticatory feeding behaviour of benthic decapods has been recognized as a special problem in feed presentation and durability (Meyers et al 1970, Meyers and Zein-Eldin 1972). Extensive investigation of pellet binders (Meyers 1980, Heinen 1981) has produced long term physical stability, but not long term chemical integrity (Conklin et al 1978, Goldblatt et al 1979). Hence, diet formulation has tended to rely on the "shot-gun" approach (Provasoli 1976), i.e. speculative inclusion of a wide range of macro, micro and trace ingredients, and the "band-aid" approach (D.E. Capuzzo, pers. comm.), i.e. addition of certain water soluble ingredients in excessive quantities, rather than on classical nutritional studies.

Testing of artificial rations for freshwater crayfish has been limited, employing prawn formulations (Huner et al 1974, Tarshis 1978, Huner and Meyers 1979). Both formulation and rigorous testing of diets is hindered by the natural omnivory (polytrophy) of crayfish, over a wide range of food sizes and types, including cannibalism and detritivory (Lormann and Magnuson 1978, Momot et al 1978), and complex selective and masticatory feeding processes prior to ingestion (Thomas 1978) in common with other benthic decapods.

In Australia, a recent proposal for the development of a patented system for battery culture of the large Western Australian freshwater crayfish, marron (*Cherax tenuimanus*), was questioned on the basis of availability of a suitable artificial ration. This paper reports the results of feed tests for this project carried out at the Western Australian Marine Research Laboratories during 1982-83 on, mainly, several commercial and experimental crustacean rations obtained from overseas. This expedient approach assumed that general nutritional requirements amongst prawns, lobsters, and crayfish are similar, an initial assumption employed previously (e.g., Huner et al 1974, Conklin et al 1975, Wickins and Beard 1978) and considered to be reasonable in view of the recognized commonality of the food problem for these decapods (Conklin 1980, Capuzzo 1981). Tests were carried out in experimental batteries, of a novel design, in which extraneous sources of possible supplemental food were minimal and cannibalism was excluded. Therefore, these results on bare substrates should be clearly distinguished from those

obtained elsewhere in ponds, tanks, and aquaria where results are confounded by the presence of natural substrates accumulating detrital and other organic material and, in groups, cannibalism. Superior growth can be expected under these latter conditions (Bovbjerg 1956, Conklin et al 1977, Mills 1979) which prevent meaningful interpretation of data (Zein-Eldin and Meyers 1973). Considerable attention has also been given to other aspects of the methodology of such food tests (e.g., New 1976) which have been often open to considerable criticism (Conklin 1980). Readily appraised methods of rating crayfish performance have been developed, both for comparing tests and, more importantly, in relation to desirable industry goals.

Comparison of different foods may appear to be straightforward at first sight, but analysis of the results will be complicated by a number of other factors which need to be taken into account in making the final assessment. For example these factors include the environmental suitability of the battery and water supply for long-term survival and the effect of close confinement.

II METHODS

A. BATTERIES

Two batteries were employed, each housing 100 crayfish, usually 0.1-5 g, in individual compartments (opaque walls), of floor area 100 x 75 mm (75 cm^2) with water depth of 45 mm and with a separate water supply inlet and bottom take-off outlet (Fig. 1). Escape of crayfish was prevented, and inspection facilitated, by close-fitting 3 mm glass covers, 500 x 75 mm, each covering five compartments and lifted by use of a rubber suction disc. Water supply from a bore in coastal limestone on the grounds of the laboratory (analysis, Table 1) was pumped through 350, 25, 5 µm and carbon filters (Cuno, AMF (Aust.)), a submerged U.V. sterilizer and heated to 22.5°C. Battery flow-through (non-recirculating) water supply was adjusted to 50, 75 or 100 changes of water per day depending on food type and crayfish size. Monitored inlet and outlet manifold oxygen levels always approached saturation. Intensity of fluorescent lighting ("Plantlife") in battery compartments under two layers of 75% shadecloth was 0.1-0.3 microeinsteins $m^{-2}s^{-1}$ (LI-185 meter, Lambda Instr. Corp., Nebraska) on a LD 12:12 daily cycle.

Some additional tests were run on larger crayfish, 5-25 g, employing round, black, opaque, plastic containers, height 18 cm, floor area, 375 (5 x 75) cm^2 (Michaelis Bayley Plastics, Melbourne) and groundwater supply at 50 changes day^{-1} and 18-22°C.

B. CLEANING AND FEEDING

Battery compartments were thoroughly cleaned (vacuum pump), and then the crayfish were fed, every two days, a compromise (interval) between a test of ration durability and the likely build-up of micro-biota. Exuviae were not removed, a standard practice (Conklin et al 1975), being consumed in preference to food within three days of ecdysis. Because of variability in the free water content of different feeds (≈10-90%), feeding rate was calculated as dry weight of feed per unit wet weight of a test

group of crayfish and recalculated weekly from observed growth (initial wet weight plus calculated ecdysial increments). The total amount fed to a group on a particular occasion was apportioned to individuals on the basis of size and proximity to recent ecdysis. For artificial rations, a feeding rate of approximately 5% per unit body weight per day was usually pursued (e.g., Conklin et al 1975, Huner and Meyers 1979, Capuzzo 1981). The actual feeding rate given in a particular test was finally calculated as a daily rate from the total dry weight of food actually fed expressed as a percentage of the mean of the initial and final measured wet weights of the crayfish.

C. FEEDS

Overseas rations were obtained soon after mid 1982 and stored frozen. Sources of these rations are given in Table footnotes in the Results, together with references to use in other feed studies and formulations; details as to closed formulations are available to varying degrees from the respective manufacturers. ICI "Pruteen" powder was alginate-bound as described below. Experimental artificial rations employed powdered Ralston Purina experimental marine ration (RP M25) as a base. RP M25 is the most widely used commercial pellet for crustacean feed although highly unstable and subject to severe "leach-out" in solution (Farmanfarmaian et al. 1982). To this base was added 7.5% soy lecithin (Nutralife), 1% cod liver oil (Faulding), 0.1% sodium ascorbate (Golden Cross), 1% multi-vitamin mix (Myadec), and an attractant 0.5% trimethyl ammonium hydrochloride (Dr B.A. Pierce, University of Hawaii at Manoa, pers. comm.). Protein content of some batches tested was increased to approximately 40% with "Pruteen" powder (75%) and Spirulina (71%, Meadow Croft). Batches were bound with 2.5% sodium alginate, either Kelgin HV (Kelco Corp, San Diego, CA) or Manugel GMB (Kelco/AIL Int., Granville, NSW) with 1% sodium hexametaphosphate (BDH) as a sequestrant. The dough mixture (≈ 55% water) was extruded (2,3 or 4 mm orifice), cut into pellets, dried partially, gelled in a $CaCl_2$ solution (0.1-2%) for 30s, oven-dried overnight at 40°C to about 20% moisture content and frozen. In practice, this alginate binding method (e.g., Meyers 1980, Farmanfarmaian et al 1982) is dependent upon partial drying before gelling and the $CaCl_2$ strength for successful gelling. Batches tested were those showing stability for two days in a simple beaker test (Tarshis 1978). Brine shrimp eggs were decapsulated as described by Royan (1980). As a standard, or reference, natural diet compost tiger worms (*Eisenia foetida*) were cultured in water-saturated peat moss and fed dried horse manure.

D. TEST CRAYFISH

Juvenile test crayfish were bred annually from domestic stock at the Pemberton Fish Hatchery, as described earlier by Morrissy (1976). About 500 newly-released juveniles were transported to Perth (23 Dec. 1981, 1981-82 year class; 11 Jan. 1983, 1982-83 year class) and held in a tank, 1.5 x 0.6 x 0.4 m, with a sand bed filter, a bunch of synthetic weed for refuge, one exchange of groundwater (18-22°C) daily and fed sparingly, weekly, on the variety of natural and artificial test feeds. Battery rearing of newly-released 1981-82 juveniles suffered

a 21% loss during the first 3 weeks due to escape and initial handling of small (<0.04 g) individuals close to ecdysis and, including the subsequent early rearing of the 1982-83 year class from 18 Feb. 1983, observation of individual ecdyses was too incomplete. Therefore, feed tests were commenced on juveniles approaching 1 g in mid July 1982 and 0.5 g in late March 1983. Groups of crayfish used in one set of concurrent tests were usually reused in subsequent tests, sometimes with an intervening period on a "reviver" diet of natural food. Pre-history of feeding was recorded for each test. Initial and final individual live weights of crayfish in each test were recorded to the nearest 0.01 g (Sartorius 1106) after a standardized drying procedure.

E. CRAYFISH PERFORMANCE

Readily appraisable test criteria were for survival, final/initial number of marron, and for the ecdysial (moult) growth increment and ecdysial frequency, two ratios developed as explained in Appendix I. The ratio for ecdysial increment, shown in subsequent tables, was the actual test weight increase due to moulting divided by the normal value from previous results. The ratio (F) for ecdysial frequency was the observed rate of moulting divided by the rate expected from the maximum growth rate of marron (Fig. 2, see Appendix I). Thus the highest growth performance in tests would be given by ratio values of 1 and poorer results would be shown by values of less than 1.

III RESULTS

A. SURVIVAL

Survival in all tests was equal, or close, to 1.00 (e.g. Tables 2,3 and 4). Usually a survival rate of over 0.90 in short term tests employing artificial feeds has been regarded as an encouraging result with other juvenile decapods. For example, by comparison to rates of 0.50, or less, shown by initial feed tests on lobsters (Conklin et al 1975). However, unlike battery cultured lobsters, short term survival of marron was unrelated to feeding as shown by a survival rate of 1.00 for an unfed control group over 100 days (Test No. 49, Table 2). While the projected estimate for annual survival over 29 tests, weighted for varying test durations, was 0.995, it is extremely improbable that this rate would be sustained for one year on the present artificial feeds, see below. These short term high survival rates are a measure of the highly favourable physical and chemical conditions provided by this battery system. In this connection, emphasis should be given to the absence of mortality, or trauma, associated with ecdysis, especially with the largest crayfish - up to 10.2 g, confined in smooth floored units of 75 cm^2 in area. Deaths almost invariably occurred amongst the smaller individuals in the test groups. For the 10 deaths in all tests to August 1983, mean size was 32% (13-55%) by weight of the test mean size. Recorded deaths would also have included any possibly associated with (rare) waterflow interruptions.

B. GROWTH INCREMENT

This performance criterion, i.e. taking a weight increment of 52% as unity, ranged down to 0.80, representing a mean individual weight increment of 22%, for commercial rations (Table 2), but was generally higher and more consistently close to unity for enhanced and bound experimental rations (Table 3) and natural feeds (Table 4). Growth increment following ecdysis has been observed previously in the field and laboratory to vary down to close to zero for individual ecdyses and may be markedly depressed for groups such as post-spawning females (Morrissy 1976) and those subject to lowered oxygen levels (Morrissy 1974). However, the present variation shown through Tables 2,3 and 4, and in association with the results for the ecdysial frequency criterion, F, (next section), appeared to be related to food types. This aspect, and the assumed value of 0.52 for the individual growth increment, was examined from the pairs of initial and final individual weights from the tests corresponding to 0,1,2, or 3 ecdyses per test. This analysis (Table 5 and Fig. 3) is detailed in Appendix II. In summary, the mean growth increment at ecdysis was approximately 52% by weight for tests in which the moult rate was more than 30% of the maximum rate, i.e. F>0.30.

C. ECDYSIAL FREQUENCY (Moult rate)

Values of the ecdysial frequency criterion, F, for commercial and overseas experimental artificial rations varied from 0.41 down to 0.07 (Table 2). The assumption, employed to derive this criterion, of a constant rate of ecdysis by each test group, was less than completely fulfilled to varying degrees on these feeds. Ecdysial frequency tended to decrease through each test with an accompanying decrease in observed food consumption. With successive ecdyses, individuals progressively lost exoskeletal pigmentation, developing a bluish appearance, and the exuviae became paler and more fragile, changes described in other species (Beattie 1972, Conklin 1976, Huner and Meyers 1979). Loss of appetite has been taken as a classical symptom of a dietary deficiency in studies of nutrition (Conklin 1976). The extreme illustration of this symptom occurred on LUV dog feed, a bound product, with a misleadingly high initial acceptance by the crayfish. Crayfish fed LUV were taken initially from the communal stock tank and tested over two consecutive 50 day periods followed by a period on earthworms (Fig. 4). Figure 4 also shows the influence of previous feeding history in inducing a certain degree of cyclic group ecdysis and the rapid response of crayfish to a favourable change in diet. Tabulation of observed ecdyses to allow ready summation of the weekly rate of ecdysis by a group during testing was later adopted as a valuable accessory criterion.

The performance of crayfish on Ralston Purina crustacean ration was not improved by lecithin, protein and vitamin enrichment and alginate binding, taking into consideration variation in container size, see later (Table 3).

A number of natural feeds were tested, of which cultured live earthworms were the most convenient and successful standard,

or reference, feed (Table 4). Together with brine shrimp, the standard, natural, control feed for lobster culture experimentation in the USA, (Rosemark 1978), worms had a high, continuing level of acceptability by crayfish at each feeding. Tests using cubes of beef liver, prawn tail meat and decapsulated brine shrimp eggs were discontinued after five days because of almost zero consumption and rapid decomposition at 22.5°C. Beef liver has been an acceptable maintenance diet for larger marron, >30 g, housed individually at <20°C (Morrissy unpublished results) but may be too fibrous for shredding by 0+-group marron. The limited and infrequent commercial availability of imported brine shrimp precluded extensive use.

Earthworms were available in sizes from 0.05 to 0.35 g so that live, whole worms could be suited to crayfish size. Fed at an overall daily rate of 1%, live, whole worms were consumed completely by intermoult crayfish within a few minutes of feeding. Worms fed on alternate days at this rate gave F values comparable to the best commercial rations fed at 5%, but rarely completely consumed. The live weight of worms fed at 1% exceeded the fresh weight of artificial rations at 5% because of dissimilar water content (Tables 2 and 4). Higher overall rates of feeding of worms could not be achieved on a two day feeding basis because of escape of live worms (or decomposition of chopped-up worms). However, by feeding worms more frequently, F values of 0.52 and 0.60 were obtained at overall feeding rates of 1.8% and 2.6%, respectively. Consumption was rarely complete and feed dry matter consumption may have been limited by the large bulk due to the high water content of worms and other natural feeds (Table 4). In practice, rates of feeding on natural feeds via more frequent feeding were also limited logistically; for feeding twice daily, 82% of the potential number of feeds were carried out and for three times daily, only 61%. These results were sufficient to support the contention that the present artificial feeds were deficient nutritionally, or became so in solution.

E. LIMITING FACTORS

(i) Confinement

However, besides a nutritional deficiency some other limiting factor may have been present in these tests, possibly battery confinement identified previously for crayfish and lobsters (Shleser 1974, Goyert and Avault 1978, Van Olst and Carlberg 1978). This factor has been commonly expressed as container floor area (Shleser 1974), i.e. physical confinement, rather than as water volume, i.e. water quality. This aspect of water exchanges per unit time has not been investigated in relation to unit size (D.E. Conklin pers. comm.). In the present study, water exchange rate was set very high for this reason and was increased with increase in crayfish size through the tests. In lobsters and other crayfish, mortality has been reported as a consequence of increasing confinement often before reduction in growth is apparent (Goyert and Avault 1978, Wickins 1982) but this trend could not be implicated in these marron studies. With increasing confinement with

increase in crayfish size, a decrease in the individual growth increment might be expected due to physical impedance during ecdysis. However, there was no tendency for a negative relationship between the growth increment ratio W_{i+1}/W_i and size of crayfish over the individual size range 0.1 to 20 g in 75 cm^2 units. For ecdysial frequency, a direct test between two groups of crayfish, of similar sizes and fed the same food, gave F values of 0.29 and 0.37 for unit areas of 75 cm^2 and 4 x 75 cm^2, respectively (Test Nos 22 and 23, Table 3). These F values were not significantly different (P=0.11) but with a larger sample size, i.e. 33 instead of 20, may have been just significant at the 5% level since the variances were homogeneous (see later statistics). Comparison of other tests, not run concurrently, where container size was varied, indicated a similar small influence (Table 3). If this factor is multiplicative, rather than additive, its influence could be of major importance at F values exceeding 0.5, but this possibility is not indicated by comparison of test Nos 5 and 26 (Table 4).

The unfavourable influence of increasing confinement with growth in size has been shown to operate from a small initial size of crayfish and not as a threshold (Goyert and Avault 1978, Richards and Wickens 1979). Shape of the floor area apparently is not influential (Schleser 1974). However, attempts have been made to quantify the relationship container area, CA, to total body length, TBL, in the form $CA = (k.\ TBL)^2$. Values of k have been indicated as 3 (Van Olst and Carlberg 1978), 2 (Richards and Wickens 1979) or 1.7 (Aiken and Waddy 1978) for lobsters.

Some practical perspective on this aspect for the present study and marron battery culture can be seen by considering a likely lower limit to k, as $(TBL)^2$, i.e. k=1, and an upper limit as (antennal span, AS,)^2, i.e. k=2.4 (Fig. 5). Goyert and Avault (1978) observed that no crayfish grew larger (in TBL) than the diameter of a compartment. TBL ≈2.8 OCL(cm) and AS was measured with the antennae at right angles to the rostral length, for sizes from 0.5 to 200 g, giving AS=6.5 OCL(cm). In 75 cm^2 units k=1 corresponds to a crayfish size of 1.7 g and k=2.4. to 22.5 g (Fig. 5). Various tests conducted on crayfish below and above 1.7 g showed no marked difference which could be attributed to the presence of such a physical threshold to explain the poor growth performance. Considering that a practical density of crayfish in a battery culture may need to be greater than 20 m^{-2}, c.f. 2.5-10 m^{-2} for semi-intensive communal pond culture (Morrissy 1979), at 120 g the corresponding density for k=1 is 44 m^{-2} and for k=2.4, 8 m^{-2} (Fig. 5).

(ii) Nutrition

Within the framework of tests employing commercial and, more especially, experimental artificial rations (Tables 2 and 3), various nutritional factors varied, or were varied, and some additional tests were carried out seeking a marked improvement in F values, i.e. to greater than 0.50.

Stock Ralston Purina M25 fed twice daily instead of every two days did not yield a significant increase in F value (Tests 8 and 11, Table 2). These pellets are subject to rapid physical disintegration in solution (Farmanfarmaian and Lauterio 1979). In addition, if decomposition, or leaching, in solution caused deterioration in the integrity of the ration, an increase in performance might have been expected, although first order leaching is extremely rapid (Goldblatt et al 1980, Cuzon et al 1982). An experimental ration coated to reduce leaching had an extremely low level of acceptability to the crayfish (Test 10, Table 2). The so-called "band-aid" approach to offsetting leaching losses by heavy vitamin supplementation (D.E. Capuzzo, pers. comm.) was also ineffectual (Table 3).

There was considerable variability in the gross protein content of various artificial feeds, noting, especially, ICI Pruteen at 75% (Table 2). The protein level of experimentally bound Ralston Purina M25 was increased for a number of tests to approximately 40% without any marked improvement (Table 3). This commercial feed has been appraised by Farmanfarmaian and Lauterio (1979) as having a near balanced amino-acid composition. An apparently high protein requirement on artificial feeds, has been ascribed to the need to compensate for amino acid imbalance. Huner and Meyers (1979) indicated that gross protein requirements of juvenile crayfish were in the range 20-30%, from tests in the range 19.6-46.0%.

Loss of appetite during tests on artificial feeds was most likely an indication of development of a nutritional deficiency. However, the possibility that this, and the generally low acceptability of artificial feeds, was due to a behavioural habituation to an unvarying diet, apparent in lobsters (Rosemark 1978), was tested by daily feeding of three foods in rotation, i.e. brine shrimp, bound Ralston Purina, and worms (Table 6). Overall feeding rate was reduced, perforce, to 1.9%, although these foods were fed to their respective, practical, daily limits in 75 cm^2 units. There was no marked increase in growth rate although crayfish fed previously on brine shrimp showed a significantly higher F value than those fed previously on artificial feed alone.

A noticeable effect of feeding live earthworms to marron was their retention of exoskeletal (shell) pigmentation. Marron fed on food of wholly animal origin, e.g. beef liver, lose pigmentation, becoming a pale bluish colour, through several ecdyses similarly to those fed formulated food. This suggested that plant detritus in the gut of worms, could provide shell pigment. The primary feeding of crayfish as detritivores in nature also suggested the desirability of testing some form of detritus - as a supplement to artificial diets - although the culture of detritus is difficult (Newell and Fell 1975). Various tests were carried out using bed material taken from worm culture boxes. A substrate of a layer of 3, or 5, mm dia. glass beads was provided in each crayfish unit. About 5 g fresh

weight of the "detritus" was added to each unit weekly, after suction removal of most of the remaining previous material. Intact, pelletted feed was removed every two days, without disturbing the substrate, before refeeding.

The first set of concurrent tests on crayfish, taken from the stock tank, yielded an F value for detrital supplementation of 0.56 but this value was not significantly different from that for pellets alone (variances homogeneous) (Tests 29 and 30, Table 7). The second set, (Tests 34 and 35) almost contemporary with the first, above, employed a smaller size of glass beads as substrate and gave an F value of 0.69 which was significantly higher than that for pellets alone ($P<0.001$, variances non-homogeneous). A third set (36 and 37), continuing with the same crayfish, showed a low growth rate on either pellets or detritus, alone. All these tests employed the same worm box material, of 3-5 months age, composed initially of soaked peat-moss and dry manure (1:1) with the worm bed fed subsequently with dry manure, sparingly at weekly intervals. The subsequent set of tests (38-1) and (39-1) employed a fresh worm bed mix of 100% manure in an endeavour to heighten the effect of detrital supplementation. However, the opposite effect occurred ($F=0.27$); over the first four weeks of the tests crayfish performed somewhat better on the supplemented diet but then their weekly rate of ecdysis declined markedly (Fig. 6). This poor performance persisted until the last of seven weeks in subsequent tests (38-2 and 39-2) where both previous groups were fed worms (Fig. 6). These results indicated a chronic toxicity derived from the enriched "detritus"; sulphide staining of the PVC outlets and odour was noted.

A most noticeable result of these tests was retention of exoskeletal pigmentation by crayfish fed detritus, in contrast to those fed artificial pellets alone. Even more striking was the regaining of near normal, dark coloration by crayfish initially pale after previous feeding solely on artificial food. This change occurred in test 39-1 following one ecdysis.

The worm and detrital diets and other factors supported the rating of marron performance on artifical food as poor. However, unlike the growth increment criterion it was not possible to validate fully the absolute basis for F values, i.e. the maximum growth rate curve, but some partially supporting analyses are detailed in Appendix III.

IV DISCUSSION

In an applied context, the present results confirm the inadvisability of capital intensive development of battery culture of marron at the present time - at least on the basis of availability of a suitable artificial food, or feeding method. As support for this conclusion, attention should be drawn to the intensive research effort, over more than a decade, on *H. tasmanicus* with the aim of establishing commercial battery

culture. Van Olst et al (1980) observed in review that - "The lack of a suitable artificial food ration is the greatest single deterrent to commercial lobster farming at present." - and others have expressed similar concern (Conklin 1978, Richards and Wickins 1979). This applied problem has been less acute for commercially important prawns and freshwater crayfish; extensive or semi-intensive pond culture circumvents direct, or total, reliance on any artificial feed employed (Conklin 1978). (However, the magnitude of research efforts on artificial diets for prawns greatly exceeds that for other decapods (New 1980)).

In a research context, aside from evident problems of feed testing methodology, there appears to be a common problem hindering all the considerable efforts to date to define the nutritional requirements of benthic decapods. In reviewing lobster nutrition, Conklin (1980) emphasized the paucity of knowledge for decapods as a reflection of this general problem.

Obscuring this problem is the difficulty of rating growth performance in many studies where feeds are compared. As shown in the present study, statistically significant differences in growth performance can be demonstrated by suitable choice of sample number and test duration; however, the highest performance on artificial feed was mediocre when objectively rated. The present results for marron on a range of artificial feeds are remarkably similar in this regard to those presented by Conklin et al (1975) from similar initial tests on lobsters. In the latter tests, weight increases on artificial feeds ranged up to 40% compared with that on live brine shrimp which was considered to give a desirable (if not maximum) growth rate. Subsequent tests on more recent artificial lobster diets appeared to yield only minor improvement in growth over the best of the initial diets when so rated (Conklin et al 1980), but D'Abramo et al (1984) appeared to have achieved a marked increase (~80%). Similarly, Huner and Meyers (1979) rated the growth of *Procambarus clarkii* on artificial diets in battery tests as comparable to that of populations under austere natural conditions.

Because of the growing academic and commercial interest in the Australian freshwater crayfish fauna (Morrissy 1984), it is opportune to bring "the crustacean food problem" to the attention of both researchers and culturists. Implications for conducting and reporting growth studies in laboratory aquaria are obvious. Of equal concern is the use of crayfish for bioassays for which it is essential to define and maintain a standard physiological condition (Seidel et al 1980). For culturists there are important implications in management, for cost-effectiveness, presented by the choice of pond feeds ranging from low cost agricultural feed and by-products to more expensive formulated feeds.

V CONCLUSIONS

(i) The experimental system - of individually housing of marron, treatment of the water supply and regular cleaning - provided for rigorous testing of the nutritional value of artificial feeds.

(ii) The battery employed, while very satisfactory for research purposes, should not be taken as a prototype for any large commercial unit. For example, a large amount of labour was involved in manual feeding and cleaning and the recording of the progress of the marron even for two hundred individuals.

(iii) Survival was very high in these short term tests. However, the survival rate of 100% for a control (unfed) group over 100 days showed that this result was unrelated to feed but rather to the excellent conditions provided by the research battery. Use of the present artificial feeds over a longer term would probably result in excessive mortality due to nutritional deficiencies.

(iv) The normal growth (moult) increment of marron, 52% by weight from previous results, occurred in tests where the moult rate exceeded 30% of the maximum rate.

(v) The moult rate was less than 50% of the maximum rate on all artifical feeds and very low on some. Live earthworms, fed frequently, and detrital supplementation of artificial feeds produced higher moult rates although feeding on these diets was limited by various factors.

(vi) Close confinement of marron in battery units did not appear to affect survival, moult increment or the moulting process. This result may have been due to the high rate of water exchange through the battery, i.e. high water quality, particularly for oxygen levels.

(vii) Symptoms of nutritional inadequacy produced by use of artificial feeds were loss of appetite, declining moult rate and loss of shell colour and thickness over the course of a test. Detrital supplementation appeared to overcome or prevent loss of shell colour.

(viii) These tests on a range of the best known commercial and some experimental artificial feeds for Crustacea, did not identify any as suitable for intensive battery culture of marron. Until this generally recognized feed problem for Crustacea is overcome at a research level, investment of capital in battery culture is inadvisable.

VI ACKNOWLEDGEMENTS

Finance was provided under contract to the Australian Industrial Research and Development Incentives Board, Canberra in co-operation with the Department of Science and Technology, Canberra. Permission to import feed samples was granted by the Department of Health, Animal Quarantine Branch, Canberra. Assistance in obtaining samples is gratefully acknowledged from the respective feed companies and the following individuals; Dr Sam Meyers, LSU, Baton Rouge, Louisiana, USA; Dr Ken-ichi Hayashi, Shimonoseki University, Japan; and Dr Dale Leavitt, Woods Hole Oceanographic Institution, Mass., USA.

Statistical analyses were carried out by N. Caputi of these laboratories. Dr D.A. Hancock kindly read the manuscript.

E. Seach and W. Gibson constructed the test equipment and maintained the support facilities.

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TABLE 1: WATER ANALYSIS; SAMPLE TAKEN 30 JUNE, 1983.

pH		8.0
K_{25} mS m^{-1} x 10^a		1930.
T.D.S.		1040.
Total hardness		340.
Total alkalinity		150.
Ca^{2+}		82.
Mg^{2+}		32.
Na^{+}		251.
K^{+}		13.
CO_3^{2-}		<2.
HCO_3^{-}	mg/ℓ	183.
Cl^{-}		443.
SO_4^{2-}		100.
NO_3^{-}		13.
SiO_2^{2-}		17.
F^{-}		0.6
Zn (total)[b]		0.02
Cu (total)[b]		0.002
Fe (in soln)[c]		<0.05

a. 2920, 17 June 1982; 2370, 29 January 1983;

b. 17 June 1982. c. 20 December 1979.

TABLE 2: COMMERCIAL AND OVERSEAS EXPERIMENTAL ARTIFICIAL RATIONS; 50 DAY TESTS, 33 CRAYFISH, FED ON ALTERNATE DAYS, EXCEPTIONS FOOTNOTED.

TEST NO.		% DW	DAILY FEED RATE %	MEAN CRAYFISH WEIGHT g	SURVIVAL	GROWTH INCREMENT RATIO	ECDYSIAL FREQUENCY RATIO (F)
3	[1] Luv, mince	66.8	2.5	1.33	1.00	0.80	0.41[a]
9	Luv, mince	66.8	4.4	1.74	0.97	0.83	0.07[b]
4	[2] Rangen 3/8"	89.0	2.7	1.63	1.00	0.89	0.31
8	[3] RP M25	93.2	4.9	1.60	1.00	0.99	0.26
11	RP M25	93.2	5.8	1.77	1.00	1.00	0.32[c]
10	[4] RP M25, coated	90.9	5.3	2.11	1.00	0.92	0.07
16	[5] Soya, 969R	91.3	4.5	2.52	1.00	0.95	0.22
18	[6] Nippai, H.P.	88.5	4.7	2.93	0.97	0.97	0.14
19	[7] Aqualim	93.7	4.5	3.13	1.00	0.99	0.14[d]
20	[6] Nippai, C3	89.9	5.0	2.88	0.97	0.97	0.08
27	[8] Pruteen, bound	61.0	8.0	0.52	1.00	0.92	0.27[e]
28	[9] Lobster, A2	88.8	8.6	0.59	1.00	0.94	0.30[e]
49	(Control	-	0.0	4.72	1.00	0.87	0.19[a]
	(Control	-	0.0	5.07	1.00	0.98	0.00[b]

[1] Luv Petfoods, Sunshine Rd, West Footscray, Victoria 3012. (% protein = 18.0)
Ref., Professor W.T. Momot, Lakehead University, Thunder Bay, Ont. P7B 5E1. (pers. comm.).

[2] Rangen Inc. P.O. Box 706, Buhl, Idaho 83316.
"Meyers" prawn feed. Ref., Tarshis (1978), Huner and Meyers (1979). (% protein = 32.4)..

[3] Ralston Purina Co. Chequerboard Plaza, St Louis, MO 63188. Experimental marine ration. (% protein = 25.0).Ref., Fair and Fortner (1981), Farmanfarmaian and Lauterio (1979), Farmanfarmian et al (1982).

[4] Capsulated Systems Inc., P.O. Box 1351, Fairborn, Ohio 45324.
Ref., Goldblatt et al (1980).

[5] Central Soya, 1200 Bank Bldg, Fort Wayne, Indiana 46802.
Low solubility, prawn pellet. (% protein = 25.0).

[6] Nippai Shrimp Feed Inc., 3-9 Moriya-cho, Kanagawa-ku, Yokahama 221, Japan.

[7] Aqualim-GIBERNA, Vigala Centre Technique, BP6, St Andre-de-Cubzac, France. Macrobrachium ration.

[8] I.C.I. Ltd., Ag. Div. P.O. Box 1, Billingham, Cleveland TS23 1LD, U.K.
Single cell protein powder. (% protein = 75.0).

[9] Experimental lobster diet. D.F. Leavitt, Environ. Syst. Lab., Woods Hole, Oceanogr. Inst. MA. 02543. (% protein = 28.1).

[a] Test days, 1-50. [b] Test days 51-100. [c] Fed twice daily. [d] 42 day test.

[e] 10 crayfish, 42 day test, unit area 2 x 75 cm^2.

TABLE 3: EXPERIMENTAL ARTIFICIAL RATION, ALGINATE-BOUND RALSTON PURINA M25 PLUS SUPPLEMENT (SEE METHODS); FEEDING FREQUENCY, ALTERNATE DAYS IN ALL TESTS.

TEST NO.	PERIOD, SAMPLE (DAYS) NO.	DAILY FEEDING RATE (%)	MEAN CRAYFISH WEIGHT g.	SURVIVAL	GROWTH INCREMENT RATIO	ECDYSIAL FREQUENCY RATIO (F)
15	50, 33	2.5	2.76	0.97	0.98	0.26
22	50, 20	2.2[a]	4.32	1.00	1.02	0.29
23[1]	50, 20	2.2[a]	4.49	1.00	0.97	0.37
29[2]	42, 10	7.0	0.59	1.00	0.90	0.42
34[3]	33, 88	6.6	0.56	1.00	1.19	0.35
36[3,4]	50, 50	5.9	0.92	0.98	0.97	0.22
38[3,5]	30, 60	4.5[a]	7.76	1.00	0.99	0.36
39[3,5]	33, 36	4.5[a]	10.51	1.00	1.03	0.33
42[3,5]	24, 24	3.7[a]	13.98	1.00	1.03	0.44
45[3,5]	32, 18	3.6	17.50	0.94	0.99	0.33

[1]Unit area, 4 x 75 cm^2. [2]Unit area, 2 x 75 cm^2.

[3]Enhanced protein content, ≈40%. [4]Poorly pound.

[5]Unit area, 5 x 75 cm^2.

[a]Mostly worm supplement.

TABLE 4: NATURAL FEEDS[1]: SURVIVAL RATIO, 1.00 IN ALL TESTS.

TEST NO.	PERIOD, (DAYS)	SAMPLE NO.	DAILY FEEDING RATE (%)	MEAN CRAYFISH WEIGHT g	GROWTH INCREMENT RATIO	ECDYSIAL FREQUENCY RATIO (F)	FEEDING FREQUENCY
2 worms	50,	33	0.7	1.04	0.91	0.30	alternate days
12 "	36,	33	1.0	2.35	1.00	0.24	
13 "	28,	33	1.0	2.21	0.97	0.32	
14 "	28,	33	0.8	2.57	0.98	0.27	
5 "	25,	33	1.8	2.52	0.94	0.52	twice daily
26 "	42,	10	2.6	0.69	0.98	0.60	2-3 x daily[a]
17 brine shrimp[2] others[1], see text.	50,	33	2.3	2.95	0.98	0.30	alternate days

[1]Dry weight (%). Worms 13.6, bring shrimp 11.0, beef liver 29.1, cooked prawn tail meat 17.5, whole 0+ marron ≈ 21.5.

[2]Frozen, San Francisco Bay Brand, Newark, CA 94560.

[a]Unit area, 2 x 75 cm^2.

TABLE 5: GROWTH INCREMENT: REGRESSION COEFFICIENT FOR FINAL WEIGHT ON INITIAL WEIGHT AND EXPECTED COEFFICIENTS FOR 1, OR MORE, ECDYSES BASED UPON A GROWTH INCREMENT OF 0.52 BY WEIGHT.

SAMPLE NO.	NO. OF ECDYSES	COEFFICIENT (± S.E.)	EXPECTED VALUE
259	0	1.045±0.0025***	1.00
809	1	1.493±0.0043***	1.52
159	2	2.211±0.0295***	2.31
11	3	3.356±0.2182 n.s.	3.51

***, P<0.001

TABLE 6: DAILY FEED VARIATION: 99 CRAYFISH WERE FED ONE OF THREE RATIONS EACH DAY IN ROTATION: BRINE SHRIMP, BOUND AND ENHANCED RALSTON PURINA M25[a] AND WORMS. OVERALL DAILY FEEDING RATE 1.9%[b]. PERIOD 50 DAYS: SAMPLE NUMBER, THREE SUBGROUPS OF 33 WITH DIFFERING PRIOR HISTORIES OF FEEDING.

TEST NO.	PRIOR TEST FOOD, FEEDING RATE, AND F VALUE	SURVIVAL	GROWTH INCREMENT RATIO	ECDYSIAL FREQUENCY RATIO (F)
31	Bound PP M25; 2.3%, 0.26	[illegible]	[illegible]	0.22*
32	Soya, 969R; 4.5%, 0.22	1.00	1.03	0.29
33	Brine shrimp; 2.3%, 0.30	1.00	1.05	0.35*

[a] Units cleaned every second day before feeding PP M25.

[b] Worms: brine shrimp: PP M25 - 1:2.4:4.6 (dry weight).

* 0.35 >0.22, P <0.01 (ANOVA).

TABLE 7: DETRITAL SUPPLEMENTATION. D ≡ DETRITUS. E ≡ BOUND AND ENHANCED RALSTON PURINA M25. CONCURRENT TESTS BRACKETTED. TESTS 29 AND 30, 5 MM DIA. GLASS BEAD SUBSTRATE; OTHER TESTS, 3 MM DIA. BEADS.

TEST NO.	DIET	DAILY FEEDING RATE (E)	MEAN CRAYFISH WEIGHT (g)	PERIOD (DAYS)	SAMPLE NO.	SURVIVAL	GROWTH INCREMENT RATIO	F RATIO	
29[a]	E	7.0	0.59	42	10	1.00	0.90	0.42	n.s.
30[a]	E+D	4.9	0.79	42	10	1.00	0.89	0.56	
34	E	6.6	0.56	33	90	1.00	1.19	0.35	P<0.001
35	E+D	6.0	0.72	20	10	1.00	1.07	0.69	
36	E	5.9	0.92	50	50	0.98	0.97	0.22	
37	D	-	0.88	50	50	0.98	0.88	0.15	
38-1	E	4.5	1.31	50	50	0.98	0.94	0.21	
39-1	E+D	4.5	1.47	50	50	0.94	0.99	0.27	
38-2[a]	worms	1.1	2.28	50	25	0.96	1.01	0.27	
39-2[a]	worms	1.1	2.31	50	25	1.00	0.95	0.14	

[a] unit area, 2 x 75 cm^2

TABLE 8: SAMPLE NUMBERS FOR VALUES OF 95% CONFIDENCE LIMITS (±) FOR F (n_1) AND THE MINIMUM DIFFERENCE FOR SIGNIFICANCE BETWEEN F VALUES AT THE 5% LEVEL OF PROBABILITY (n_2).

	n_1	n_2
.02	144	288
.03	64	128
.04	36	72
.05	23	46
.06	16	32
.08	9	18

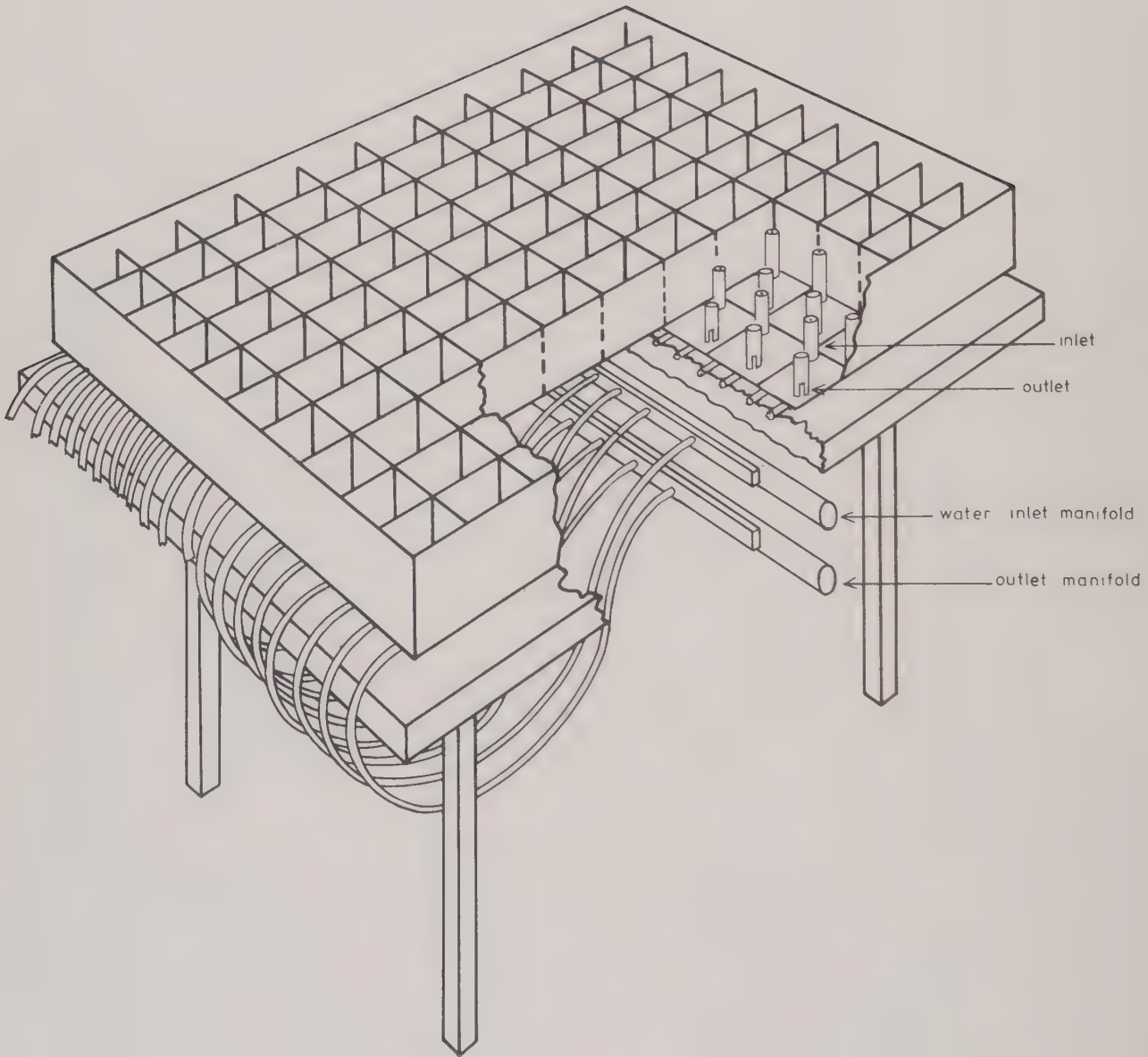

Fig. 1 Battery design

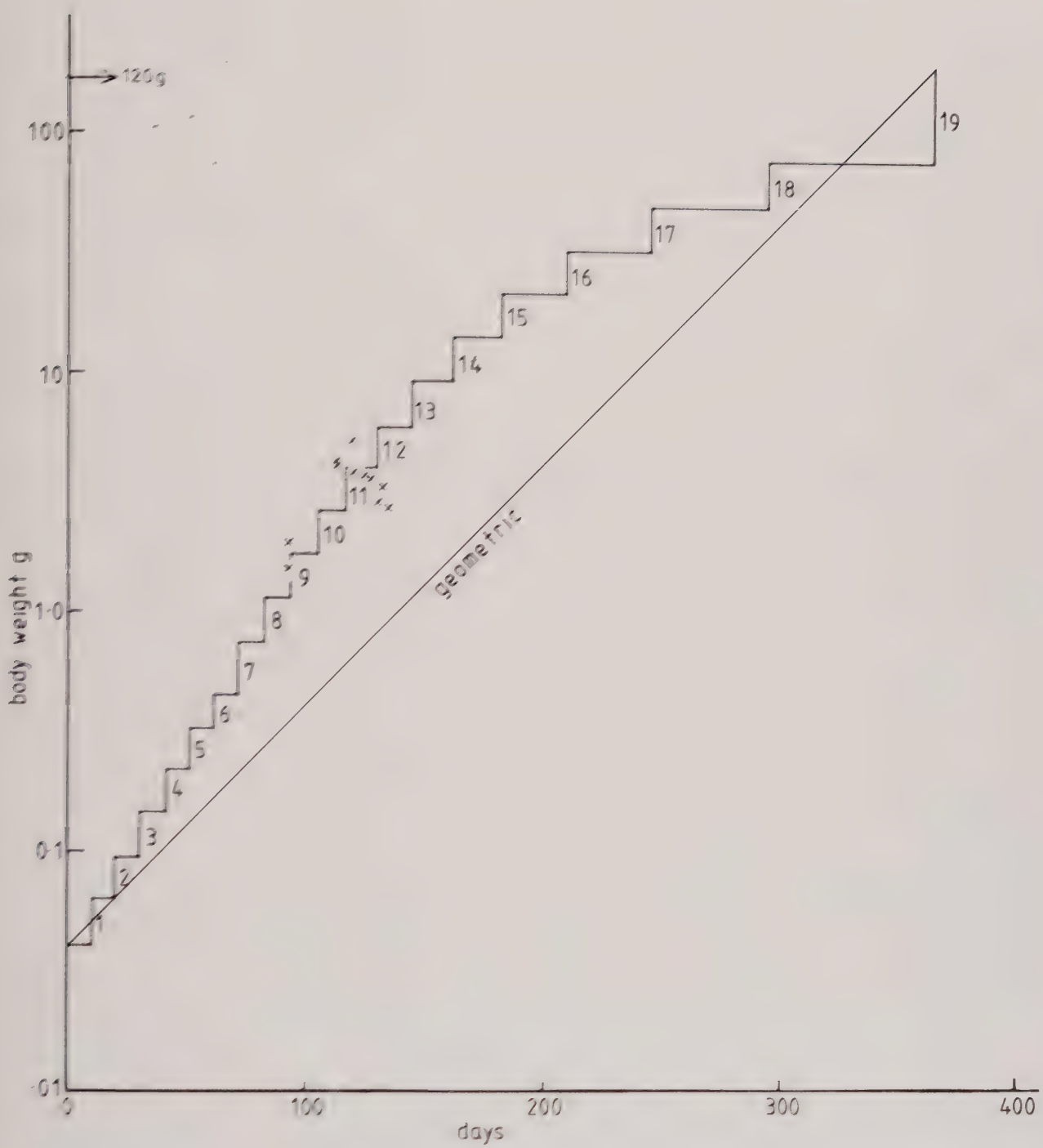

Fig. 2. Derived curve for maximum growth rate of *Cherax tenuimanus*.
x mean size for pond-cultured year-classes.

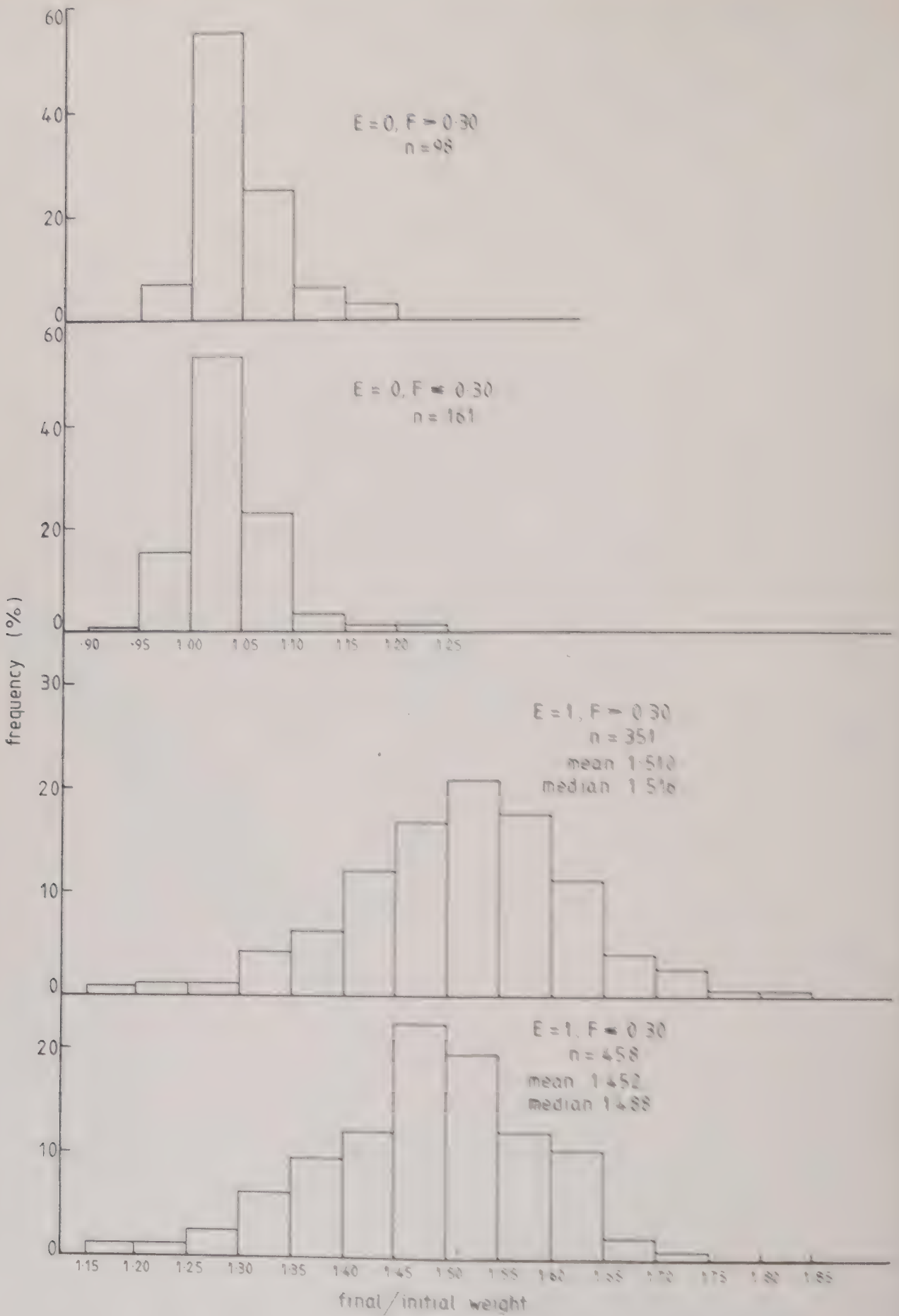

Fig. 3 Histograms showing the frequency of test values for final/initial weight for individuals ecdysing zero (E=0) and one time (E=1), grouped for test ecdysial frequency ratio values F≤0.3 and >0.3.

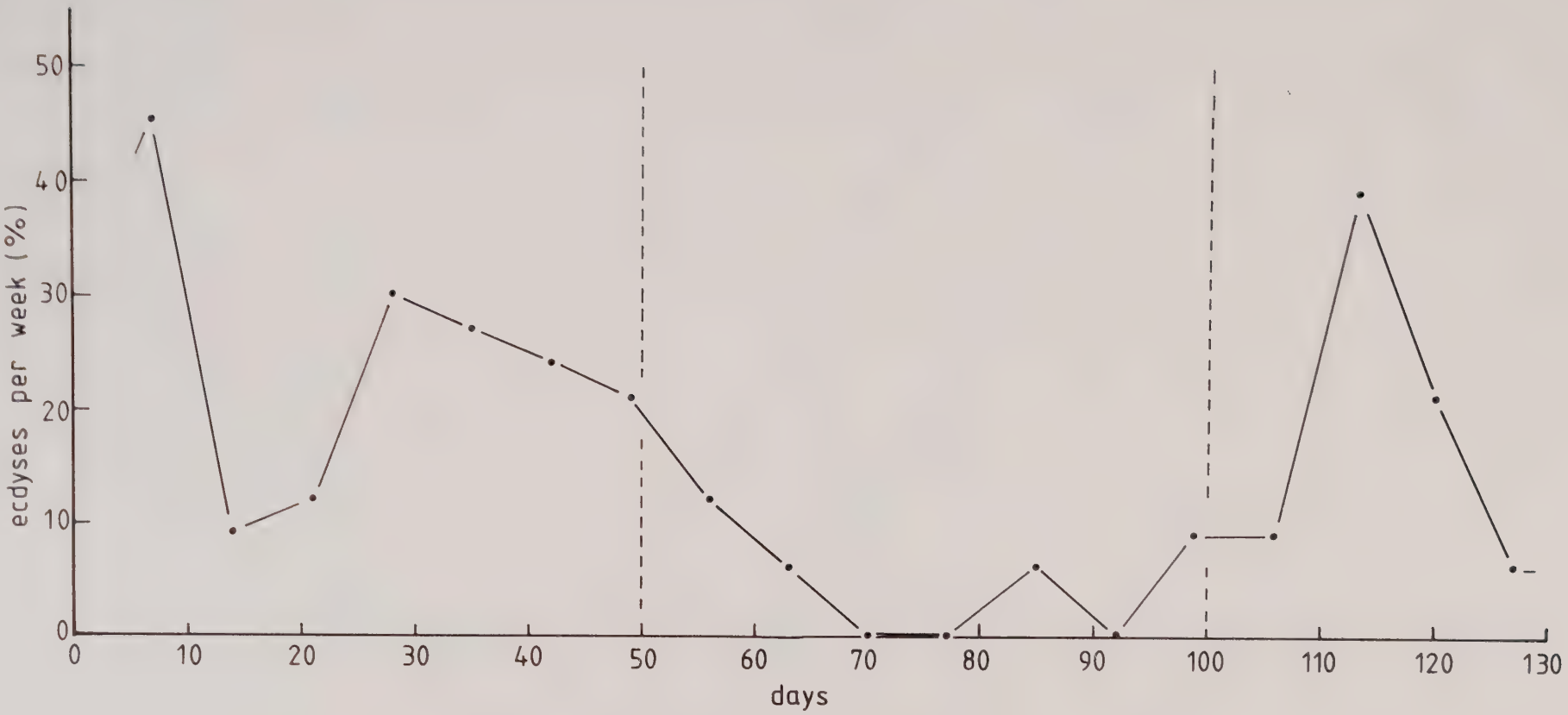

Fig. 4 Frequency (%) of ecdysis per week amongst test crayfish fed LUV in test no. 3, 1-50 days, and test 9, 51-100 days, followed by earthworms in test 13. Crayfish initially from a stock tank.

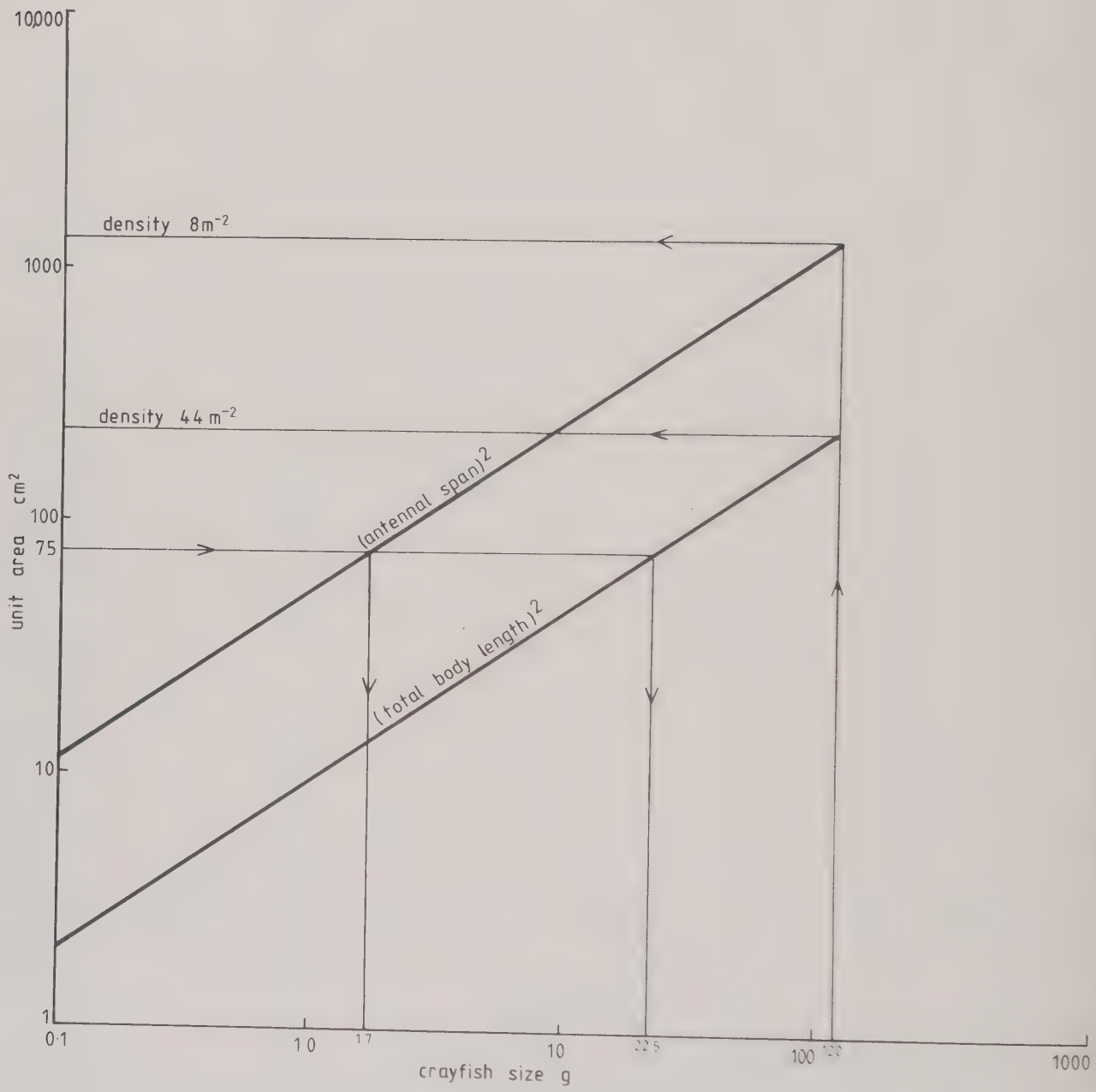

Fig. 5. Relationships between battery unit area and crayfish size based upon (total body length)^2 and (antennal span)^2.

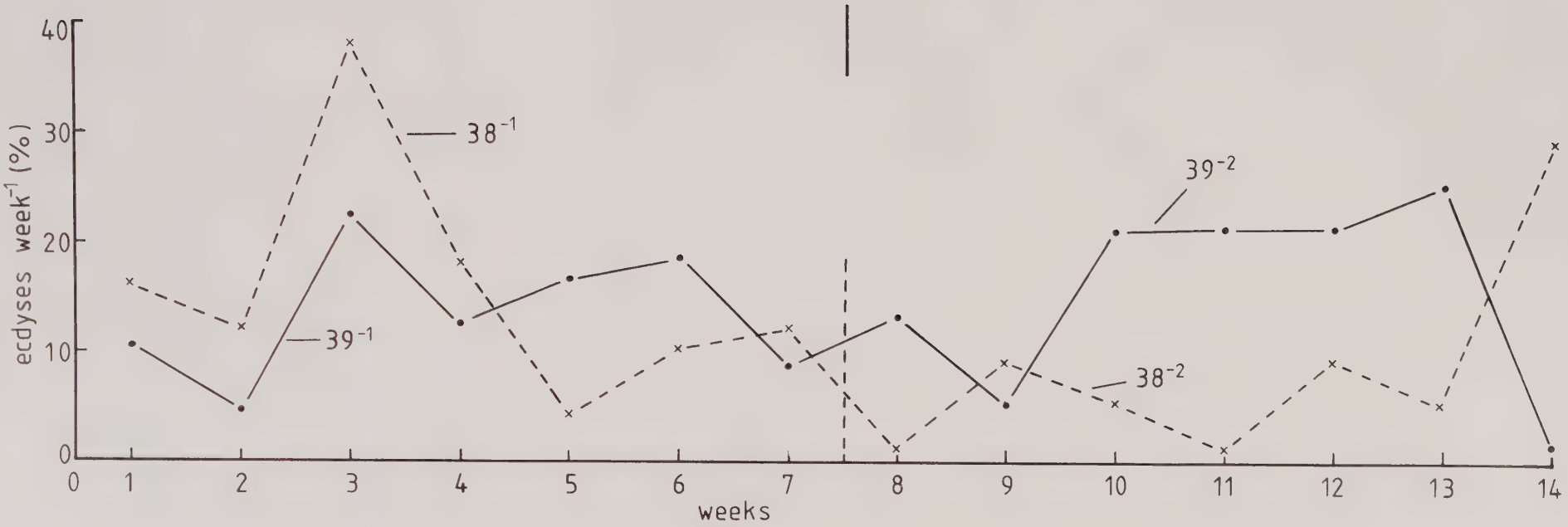

Fig. 6. Frequency (%) of ecdysis per week amongst two test groups of crayfish fed artificial food plus detritus, test no. 38-1, and artificial food solely, test no. 39-1, for seven weeks, followed by earthworms for both groups, test nos 38-2 and 39-2, respectively.

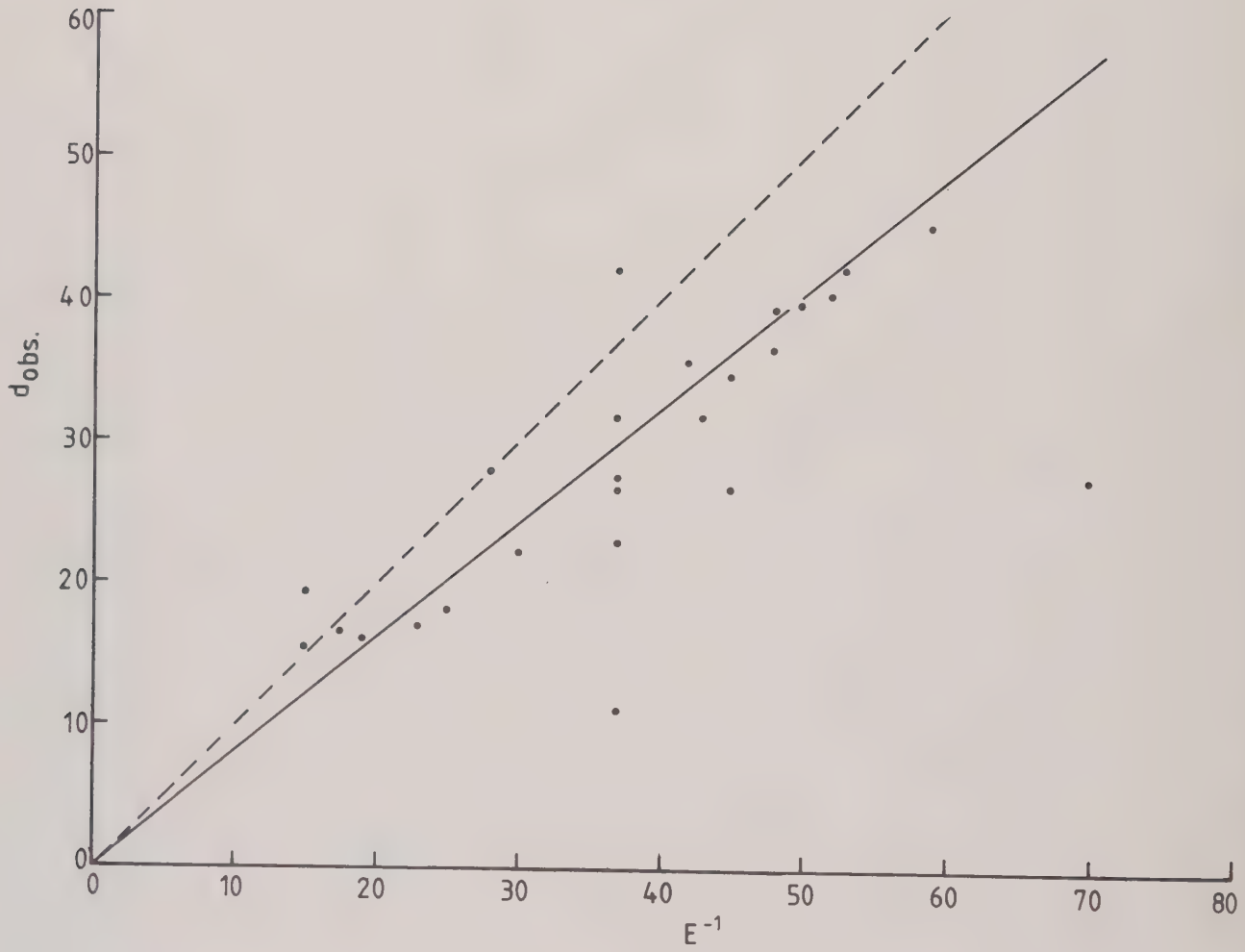

Fig. 7. Relationship between the mean, observed, test, intermoult duration, d_{obs}, and its estimate, the reciprocal of the number of recorded ecdyses per week per marron, E^{-1}.

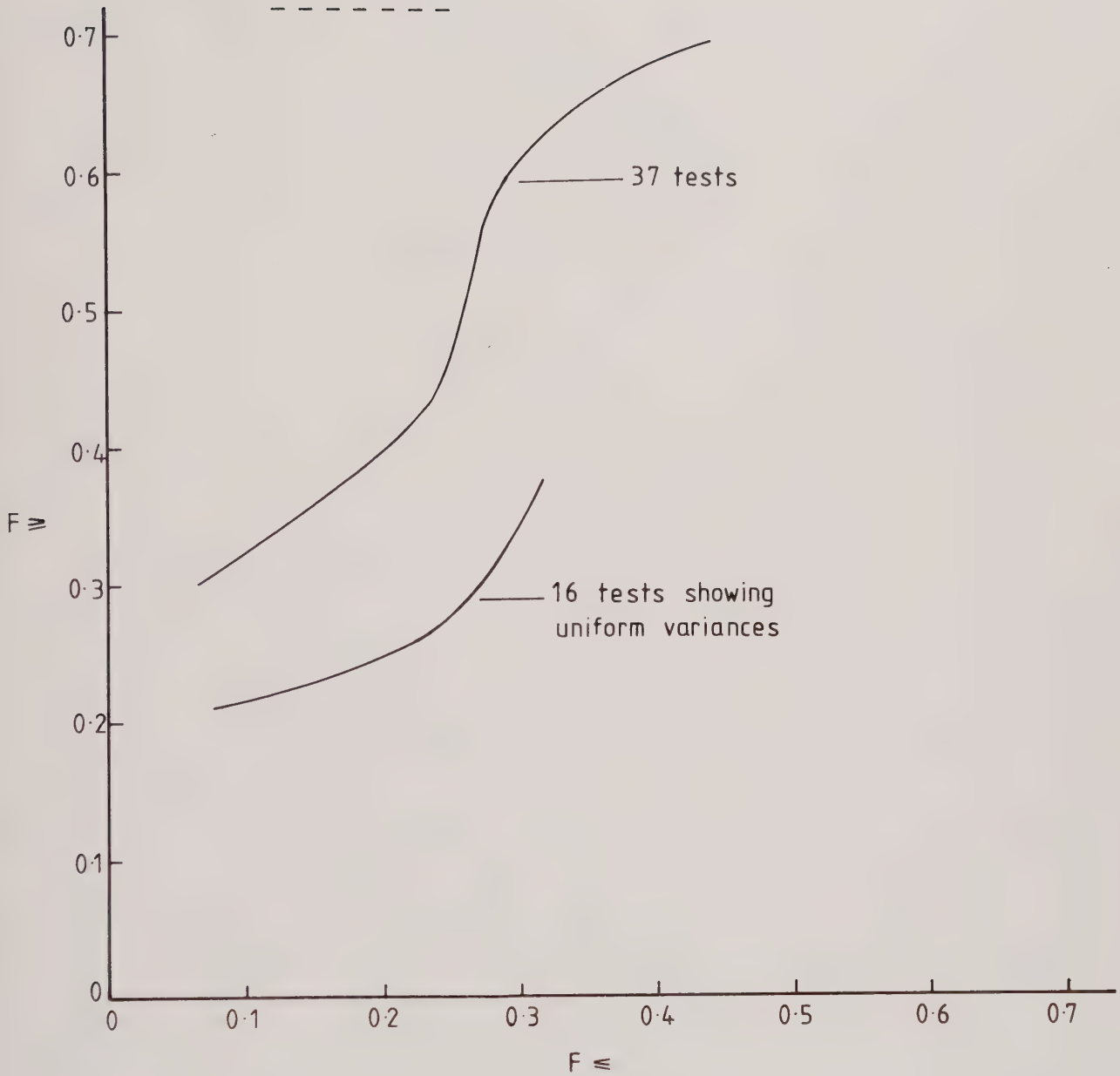

Fig. 8. Empirical curves (freehand) derived from Multiple RAnge Tests on values of the ecdysial frequency ratio, F. Curves represent boundaries distinguishing F values, significantly different at the 5% level of probability.

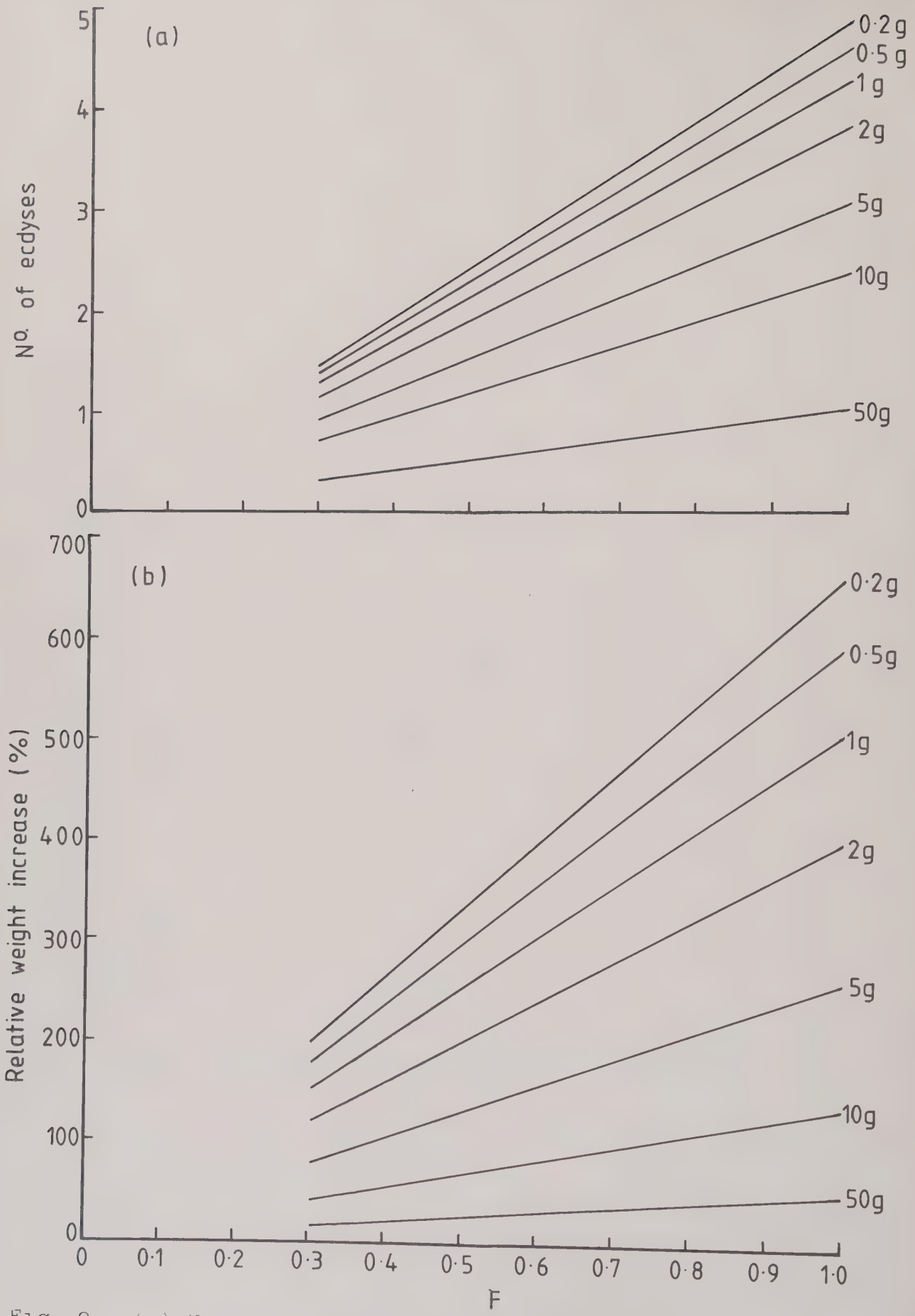

Fig. 9. (a) Number of ecdyses and (b) relative weight increase (%) per individual corresponding to F values for 50 day tests. An ecdysial increment of 0.52 by weight was employed for F>0.3.

APPENDIX I - Growth performance ratios

Available moult increment data, 0+-group (0.4-7.5 g, n = 120) and 1+-group (13-113 g, n = 57), consisted of measurements of eye-orbit carapace length (OCL), l_i cm, for successive intermoults, i, on exuviae (0+) or individuals (1+) from marron housed individually at 20°C and fed natural foods (Morrissy, unpublished results). These gave the relationship $l_{i+1} = 1.1540\ l_i - 0.0039$ ($r = 0.47$, $P<0.001$), constrained to a realistic origin of (0.40, 0.35), for the first post-release ecdysis observed from sample length-frequency distributions of pond-bred marron (Morrissy 1976). This so-called Hiatt equation (Kurata 1962) has been used in preference to a logarithmic one (Mauchline 1977). Length was converted to body weight, w_i g, using the equation $w_i = 0.74068\ l_i^{2.98932}$ revised from that used previously for marron (Morrissy 1970) by addition of data down to 0.10 g (-578 g, n = 381) with the carapace length of 0+-group marron smaller than 0.6 g (weighed to 0.001 g) measured micro- scopically (x12) rather than by vernier caliper. From the two equations above, the relative increment $\Delta w_i/w_i$, calculated by integration, increased non-linearly from 0.49 to 0.53 over the range 0.04 to 120 g. A single value of 0.52, appropriate to the test sizes of marron, was adopted to facilitate numerous individual test computations (overestimation at 0.04 g, 4%; underestimation at 120 g, 1%). A food test criterion for growth increment, with an expected value of unity, was calculated as the observed final mean weight of crayfish divided by the calculated final weight expected from a 52% increment after each ecdysis. Since the number of ecdyses for an individual was low (3 or less), ambiguity was rare in inferring unrecorded ecdyses (<10%). This criterion can be expected to vary about unity, and is linearly related to the mean individual growth increment. But where the ecdysial weight increment differs consistently from 0.52, the value of ratio depends upon the number of ecdyses, i, according to $[(1 + I)/1.52]^1$, where I is the actual increment. For an increment less than 0.52, the ratio will progressively decline through successive ecdyses.

For an ecdysial frequency (moult rate) criterion, the maximum growth rate of marron from parental release was taken as 120 g over 12 months. A mean size of 120 g over 14½ months has been recorded at very low density (1/10m^2) in a farm dam where growth was slowed over winter (Morrissy 1974). Taking 120 g as a starting point, values of Δw_1 were back-calculated through 19 ecdyses down to an initial release size of 0.04 g. The intermoult periods, d_i days, between these ecdyses, i = 1, ---19, were then calculated from the equation $d_i = d_1 + [\Delta w_i(365-19d_1)/-120]$ based upon the simplistic assumption that d_i and Δw_i (or w_1) are linearly related. The value of d_1 was taken provisionally as 10, rather than 5 or 15, based, as before, on post-release size-frequency distributions of pond marron. The resulting growth curve (Figure 2) agreed well with early pond growth of marron over the present test size range, and conformed in slope at larger sizes with previous field growth curve estimations (Morrissy 1974, 1980) which do not fit a geometric model, noting that the maximum size of marron approaches 2000 g.

For an ecdysial frequency (moult rate) criterion, F, of range zero to unity (day^{-1}) (maximum frequency), the mean number of ecdyses ($marron^{-1}$ day^{-1}) for each test, E, was divided by $1/d_i$, with d_i corresponding to the mean weight of test crayfish. That is, the mean intermoult period for each test was estimated by 1/E on the assumption that the rate of ecdysis per unit time was constant over the test period. The F values presented, and employed in Multiple Range Tests (Duncan 1955) and ANOVA (Sokal and Rohlf 1969) were calculated as above, employing the frequency distribution for number of ecdyses per individual crayfish, allowing a variance to be given to each F value.

APPENDIX II - Statistics and validation relating to the growth increment ratio

From simple linear regression analyses of final weight on initial weight, the coefficient was significantly greater than the expected value for zero ecdyses and significantly less for one and two ecdyses (Table 5). For zero ecdyses, the 4.5% weight increase over the course of a test probably is due to replacement of water uptake at the previous ecdysis by denser body tissue and organ growth during intermoult. Because of this weight gain, test growth increment ratios calculated for crayfish showing one, or more, ecdyses per test, tended to be slightly less than those reported, calculated by including crayfish which did not ecdyse. (A further factor adding to this discrepancy is the weight loss represented by the uneaten chela of the exuvium following ecdysis). These aspects highlight an interesting methodological, but in practice trivial quantitative, problem, that of defining and obtaining a true measure of growth increment.

Taking the wider-ranging test values of the ecdysial frequency criterion, F, as a more obvious measure of the influence of food type on growth increment, the intermoult weight gain was expressed by the multiple regression equation:

$$W_{i+1} = 0.067 + 1.042W_i + 0.044 \ln F$$

with the coefficient for lnF having $P<0.05$.

Similarly, for weight increments associated with one ecdysis per test, the relationship was

$$W_{i+1} = 0.255 + 1.488W_i + 0.175 \ln F$$

with the lnF coefficient having $P<<<0.001$.

This correlation between growth increment and the F values, and by inference, causally, with food type, explained the significant discrepancy between the overall mean test value for individual growth increments, i.e. 49%, and the assumed value of 52%. The scattergram of individual values of W_{i+1}/W_i against the test F value showed a broad increase in the former values up to F=0.30. Histograms for the frequency of the weight ratio value above and below the latter level clearly showed a tendency toward symmetry, i.e. a Normal response, about a growth increment value very close to 52%, above F=0.30 (mean 1.510, median 1.516) (Fig. 3). The increased intermoult weight gain can also be seen with this distinction made between food tests (Fig. 3).

APPENDIX III - Statistics and validation relating to the F values (moult rate ratio)

Partial validation of the basis for calculating the F criterion in all the tests was obtained by considering the observed mean intermoult duration, d_{obs}, for each test, i.e. based upon individuals for which two or more dates of ecdysis were recorded. This estimate was compared with the other estimate of the mean duration, E^{-1} i.e. the reciprocal of the number of ecdyses marron^{-1} day^{-1}, employed in the calculation of F. Because of individual variability in ecdysial frequency, d_{obs} was based upon crayfish ecdysing more frequently than the group as a whole; therefore, d_{obs} underestimated E^{-1}. However, for 25 tests with d_{obs} weighted for number of observations per test (1-38), there was a satisfactory linear relationship through the origin (Fig. 7). The regression coefficient was 0.80 ± 0.014 ($P<0.001$), i.e. a constant degree of underestimation of E^{-1} by d_{obs} of 20%.

In addition, the assumption of a linear relationship between the intermoult duration and size appeared to be a reasonable approximation as judged by plotting values of E^{-1} against mean test size of crayfish. The slope of this relationship at 0.87 was closer to that of 0.77 for d_{max}, the duration for maximum growth rate, against size for the assumed initial value of 10 days than for 5 (1.2) or 15 days (0.37).

While the novel use of the F criterion permitted a ready evaluation of various feeds, there was some methodological interest in the ability of these tests to provide adequate statistical comparisons, in terms of sample numbers and test duration.

From Multiple Range Tests, each subset range of actual test values of F yielded a lowest, "F<", and highest, "F>", value not overlapping with the previous and subsequent subsets, respectively. A freehand curve was drawn through plotted values of "F<" and F>" (Fig. 8). The upper curve in Figure 8 covers the full range of F values for 37 tests, including some tests of dubious validity, and not previously tabulated because of small sample number, 6-10, and short term, 30 days. Significant discrimination was barely satisfactory up to about F=0.25. However, all values up to 0.72 appeared to be significantly lower than the theoretical maximum of unity. A second Multiple Range Test was carried out on F values showing uniform variance. This test excluded higher values of F largely on the basis of higher variance (see below). The lower curve in Figure 8 showed that a reasonably fine degree of significant discrimination could be made between these tests.

Moreover, since these empirical curves were based upon actual F values, finer differences can be detected. For most of the tests of 50 day duration, the sample standard deviaton, s, was about 0.12 for F values from 0.1 to 0.4 and for crayfish up to 5 g. On these bases, approximate sample size, n, for various 95% confidence limits, CL, can be calculated from

$n \approx t^{0.975}_{n-1} s/CL^2$, where $1^{0.975}_{n-1} \approx 2$ for $n>30$,

and are shown in Table 8.

Similarly, calculation of the minimum difference for significance, at the 5% level of probability, showed that a sample number of 32 allowed discrimination between F values 0.06 apart (Table 8).

For higher values of the mean crayfish test weight, w g, up to 17.5 g, and/or higher values of F, up to 0.72, the standard deviation, s, of F values was higher; the relationship was $s = 0.0665 + 0.0133\ w + 0.189F$ by multiple regression; adjusted r square 0.76, coefficients for w and F, $P<0.001$ and $P=0.001$, respectively. Since the standard deviation of F values was based upon individual ecdysial rates, this increase with size and F is largely a reflection of heterogeneous individual growth rates. However, the coefficient of variation for F values, CV, decreased with increase in F according to the complementary relationship $CV = 17.2 - 54.7\ \ln F + 3.61\ w$.

Finally, Figure 9 allows values for, (a), mean number of ecdyses per individual and, (b), relative individual weight gain to be derived readily from F values for 50 day feeding tests.

34179/6/84—1M—L/3696

WILLIAM C. BROWN, Government Printer, Western Australia

ISBN O 7244 9317 4
ISSN 0726–0733